Quidel Corporation

QuickVue® Chlamydia
Procedure Manual

Procedure:

Prepared by	**Date Adopted**	**Supercedes Procedure #**

Review Date	**Revision Date**	**Signature**

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This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete package insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete package insert in accordance with FDA labeling regulation (21CFR809.10). Prepared in accordance with the guidelines recommended by the National Committee for Clinical Laboratory Standards, Villanova, PA 19085; NCCLS Document GP2-A2.

QuickVue® Chlamydia
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PRINCIPLE:

The QuickVue Chlamydia Test is a lateral flow immunoassay intended for the rapid, qualitative detection of chlamydia directly from endocervical swab and cytology brush specimens. The test is intended for use as an aid in the presumptive diagnosis of chlamydial infection.

Chlamydia trachomatis is the most common cause of sexually transmitted venereal infection in the world, with an incidence estimated at 3 to 4 million cases per year in the United States.[1] Chlamydia are composed of elementary bodies (the infectious form) and reticulate or inclusion bodies (the replicating form) and comprise 15 known serovars.[2]

Chlamydia trachomatis has both a high prevalence and asymptomatic carriage rate, with frequent serious complications in both women and neonates. Complications of chlamydial infection in women include cervicitis, urethritis, endometritis, pelvic inflammatory disease (PID) and increased incidence of ectopic pregnancy and infertility.[3] Vertical transmission of the disease during parturition from mother to neonate can result in inclusion conjunctivitis and pneumonia.[4]

Various methods are available for the diagnosis of chlamydial infection. Conventional isolation of *Chlamydia trachomatis* involves culturing the organism in a suitable cell line.[5]

The culture may be stained for visual examination with Giemsa, iodine or fluorescein-conjugated antibodies after 48-72 hours. More recently, rapid immunoassays using antibodies to chlamydia antigens have also been developed. These methods include direct fluorescence assays and enzyme immunoassays.

To perform the test, an endocervical clinical specimen is obtained and placed into a tube containing Reagent A (Extraction Solution); after 2 minutes, Reagent B (Neutralization Solution) is added to the tube. After extraction and neutralization, 3 drops of extracted sample is added to the Test Cassette Sample Well.

The sample migrates through a label pad containing a monoclonal anti-chlamydia antibody conjugated with a pink-colored test label and a blue-colored control label. If the sample contains chlamydial antigen, the antigen will bind to the antibody coupled to the pink-colored test label which, in turn, will bind a second monoclonal anti-chlamydia LPS antibody spotted on the membrane.

As the chlamydial antigen-antibody complex is captured, a faint-to-dark red Test Line will be visible. A blue Control Line will also appear in the Result Window indicating that proper volume of fluid entered the Test Cassette and capillary flow occurred. If chlamydial antigen is not present or present at very low levels, only a blue Control Line will be visible. If the blue Control Line does not develop, the assay is invalid.

REAGENTS AND MATERIALS SUPPLIED:

- Individually-wrapped Test Cassettes (25)
  Murine monoclonal antibodies to chlamydia (Test Line) and a control rabbit polyclonal antibody capable of binding the blue-colored control label (Control Line).

- Reagent A (1)
  0.2N sodium hydroxide

- Reagent B (1)
  0.1N hydrochloric acid

- Tubes, Tips and Disposable Droppers (25)

- Transport Tubes each with 2 sterile Dacron Swabs (on solid plastic shafts) (25)

- Positive Control (1)
  ca. 350 IFU/test formalin-inactivated chlamydia, 0.02% sodium azide

- Negative Control (1)
  ca. 10^6 CFU/test heat-inactivated group B Streptococcus, 0.02% sodium azide

- Package Insert (1)

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WARNINGS AND PRECAUTIONS:

1. Use appropriate precautions in the collection, handling, storage and disposal of the specimens and used kit contents.[6] Discard used materials in a proper biohazard container.

2. The Test Cassette must remain sealed in the protective foil pouch until just prior to use. All kit components must be at room temperature prior to use.

3. Reagent A contains sodium hydroxide – a basic solution; Reagent B contains hydrochloric acid – an acidic solution. If either of the reagents contact the skin or eye, flush with large volumes of water.

4. Use only sterile, dacron swabs or cytology brushes to obtain endocervical specimens.

5. To obtain accurate results, you must follow the Package Insert instructions.

6. The controls contain sodium azide. Sodium azide may react with lead or copper plumbing to form explosive metal azide. [Copious quantities of water should be used to flush discarded test solutions down a sink.]

KIT STORAGE AND STABIITY:

Store kit at room temperature 59-86°F (15-30°C) out of direct sunlight. Kit contents are stable until the expiration date printed on the outer box. Do not freeze.

SPECIMEN COLLECTION AND STORAGE:

The quality of the specimen obtained is of extreme importance.[7] Detection of chlamydia requires a vigorous and thorough collection technique which provides cellular material rather than just body fluids.

A first swab should be taken to remove excess mucus from the exocervix. The second swab should be inserted into the endocervical canal, past the squamocolumnar junction, until most of the tip is no longer visible. This will permit acquisition of columnar or cuboidal epithelial cells which are the main reservoir of chlamydia organisms. Firmly rotate the swab for 15 - 20 seconds. The swab should be withdrawn without contamination with exocervical or vaginal cells.

Alternatively, endocervical specimens can be collected with a cytology brush (caution: do not use cytology brushes with pregnant patients). After cleaning the exocervix with the dacron swab, insert the cytology brush into the endocervical canal past the squamocolumnar junction. Leave in place two to three seconds. Rotate the cytology brush two full turns; withdraw the brush without touching any vaginal surface.

The specimen may be tested immediately or returned to the provided transport tube for storage or transport. Do not place the specimen in any transport device containing medium since transport medium interferes with the assay and viability of the organisms is not required for the assay.

Specimens may be stored for 6 hours at room temperature (59 - 81°F, 15 - 27°C) or 72 hours refrigerated (36 - 46°F, 2 - 8°C). It is recommended that specimens be processed as soon as possible after collection.

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TEST PROCEDURE:

All reagents, Test Cassettes and clinical specimens must be at room temperature before beginning the assay.

Extraction Procedure:

- Add 5 DROPS of REAGENT A to a clean tube.

- Insert the patient swab into the tube containing Reagent A.
  ⇒ Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.

- WAIT 2 MINUTES
  ⇒ Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.

- Fill a clean disposable dropper to the bottom of the bulb with REAGENT B. With the swab shaft to the side, add REAGENT B to the tube. Discard the dropper.
  ⇒ Compress the bottom of the tube between the thumb and forefinger and twirl the swab 10 times.

- Express the liquid from the swab by compressing the middle of the tube and pulling the swab up through it. Discard the swab.

- Insert a tip onto the tube.

Test Procedure:

- Remove the Test Cassette from the foil pouch and place it on a clean, dry, level surface.

- Add 3 DROPS of the extracted sample from the tube to the round Sample Well on the Test Cassette.

- READ RESULTS AT 10 MINUTES. Some positive results may be seen earlier.

INTERPRETATION OF RESULTS:

Refer to the Procedure Card for visual color interpretation of the Test and Control Lines.

Positive Result :
The appearance of **any** faint-to-dark red Test Line next to the letter "T" in the Result Window, along with a blue Control Line next to the letter "C" indicates a positive result. A positive QuickVue result indicates that the specimen is a presumptive positive for the presence of chlamydial antigen.

Negative Result :
The appearance of only the blue Control Line next to the letter "C" in the Result Window indicates a negative result. A negative QuickVue result indicates that the specimen is a presumptive negative for the presence of chlamydial antigen.

Invalid Result :
The test result is invalid if the blue Control Line next to the letter "C" is not visible within 10 minutes. If this occurs, retest using 3 drops of the remaining extract solution and a new QuickVue Test Cassette or contact Quidel Technical Assistance.

QUALITY CONTROL:

Built-In Control Features
The QuickVue Chlamydia Test contains built-in control features. The two color result format provides a clear-cut readout for positive and negative results. The appearance of a blue Control Line next to the letter "C" provides several forms of control.

First, detection components for the specimen and internal control are processed concurrently using identical procedures; therefore, the appearance of the Control Line ensures that functional activity is maintained for both components.

Secondly, the appearance of the Control Line also ensures that the foil pouch integrity has been maintained and that the Test Cassette has been stored in such a manner as not to compromise its functionality.

Third, the appearance of the Control Line indicates that proper volume of fluid entered the Test Cassette and capillary flow occurred. This would indicate that the Test Cassette was assembled properly, by acting as a check for all membrane interfaces and proper positioning of components.

Lastly, if the Control Line does not develop within 10 minutes, the test result is invalid. As a negative control, the background color in the Result Window area should be white to light pink within 10 minutes and not interfere with the reading of the test result.

If background color remains in the Result Window which interferes with your ability to read the test result, your result may be invalid. In this case, contact QUIDEL Technical Assistance.

Positive and Negative Quality Control
External control materials may also be used to assure that the reagents and assay procedure are performing properly. For this purpose Positive and Negative Control solutions are supplied in the kit.

Add two drops of the Positive or Negative Control solution to a sterile dacron swab; allow the drops to be absorbed into the swab. Continue with the assay as described in the Test Procedure using these swabs in place of a patient specimen.

The QuickVue Positive and Negative Control solutions should be tested with each new lot or shipment of test materials once for each 25-test kit and as otherwise required by your laboratory's standard quality control procedures.

If controls do not perform as expected, do not use the test results. Repeat the test or contact QUIDEL Technical Assistance.

LIMITATIONS:
The QuickVue Chlamydia Test has been tested using endocervical swab and cytology brush clinical specimens for the qualitative detection of chlamydia antigen. Performance with other specimens has not been assessed.

The test employs genus-specific monoclonal antibodies and will not specifically differentiate C. trachomatis, C. pneumonia or C. psittaci.

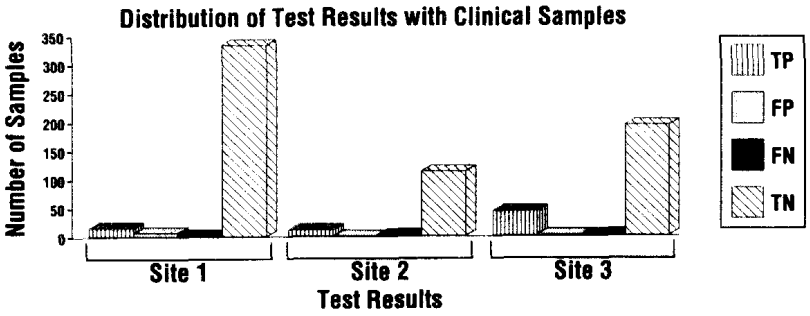
Detection of chlamydia is dependent on the number of organisms present in the specimen. This may be affected by specimen collection methods and patient factors such as age, history of STD, presence of symptoms, etc. The minimum detection level of this test may vary according to serovar.

Test results should be interpreted in conjunction with other laboratory and clinical data available to the physician. Standard chlamydial cell culture methods should be used in the evaluation of suspected sexual abuse and for other medicolegal cases where diagnosis could lead to adverse psychosocial impact.

Spermicides, gynecological lubricants and talcum powder were evaluated in the test and did not affect the expected results at concentrations up to 4.5 mg/test. The presence of mucus and whole blood in cervical specimens does not have an inhibitory effect on the test. However, grossly bloody specimens (approximately 100µL whole blood/test) may interfere with your ability to read the test result and therefore invalidate the result.

Therapeutic failure or success cannot be determined as antigen may persist following appropriate antimicrobial therapy.

EXPECTED VALUES:
In high-risk populations, such as women attending STD (Sexually Transmitted Disease) clinics, sexually active women less than 25 years of age, women confirmed with Neisseria gonorrhoea infection, or those exposed to a partner with a sexually transmitted disease, the prevalence of chlamydia infection has been reported to be between 10 and 20%. In a low risk population, such as those patients attending obstetrics and gynecology practices, the prevalence of chlamydia infection is estimated to be approximately 5% or less. The prevalence of chlamydial infection by culture in the multi-center clinical study presented below ranged from 4.4% to 17.9%. A histogram showing the distribution of 723 clinical specimens is shown below. Description of this study with explanations of interpretation of test results and result resolution with Direct Fluorescent Antibody (DFA) is included in Performance Characteristics.



PERFORMANCE CHARACTERISTICS:

Clinical Sensitivity, Specificity and Accuracy

A total of 723 endocervical specimens, obtained from women attending sexually transmitted disease (STD) clinics, Family Planning and OB/GYN clinics, were evaluated in a multi-center clinical study. Testing was performed by users with various levels of work experience and educational backgrounds.

Clinical performance characteristics were determined for the QuickVue Chlamydia Test relative to culture techniques, both before and after resolution of QuickVue positive/culture negative specimens by DFA analysis.
Seventy-three (73) endocervical specimens were positive by culture and 67 of these were also positive by QuickVue Chlamydia Test.

Of the 73 culture positive specimens, the QuickVue test correctly identified 81% (13/16) of the 1+ cultures corresponding to approximately ≤100 IFU/mL; 91% (21/23) of the 2+ cultures corresponding to 100-1,000 IFU/mL; 97% (28/29) of the 3+ cultures corresponding to 1,000-10,000 IFU/mL; and 100% (5/5) of the 4+ cultures corresponding to >10,000 IFU/mL.

The results summarized in Table 1 contains a comparison of the QuickVue test to culture and DFA.

Of the 11 QuickVue positive/culture negative specimens, 2 were found to be positive upon analysis by DFA and an additional 3 were positive by PCR*, indicating that the 5/11 QuickVue false positive results were, in fact, true positives that were missed by culture.

* FDA-Cleared Amplicor Polymerase Chain Reaction (PCR) Test

TABLE 1
PERFORMANCE SUMMARY

Culture		POS	NEG	NEG	POS	NEG				
QuickVue Test		POS	POS	POS	NEG	NEG				
DFA**		ND	POS	NEG	ND	ND				
(Interpretation)***	Prevalence %	(TP)	(TP)	(FP)	(FN)	(TN)	Sensitivity* %	Specificity* %	PPV %	NPV %
Site 1 Asymptomatic	4.8	12	0	4	2	271	85.7 (57.2 - 98.2)	98.5 (96.3 - 99.6)	74.2	99.3
Site 2 Asymptomatic	9.5	7	0	0	0	67	100 (59.0 - 100)	100 (94.6 - 100)	100	100
Site 3 Asymptomatic	17.6	24	1	0	1	116	96.0 (79.6 - 99.9)	99.1 (95.3 - 100)	95.8	99.2
Total Asymptomatic	9.1	43	1	4	3	454	93.6 (82.5 - 98.7)	99.1 (97.8 - 99.8)	91.2	99.4
Site 1 Symptomatic	4.4	3	0	3	0	62	100 (29.2 - 100)	95.4 (87.1 - 99.0)	50.0	100
Site 2 Symptomatic	11.5	4	0	0	2	46	66.7 (22.3 - 95.7)	100 (92.2 - 100)	100	95.9
Site 3 Symptomatic	18.4	17	1	2	1	77	94.4 (72.7 - 99.9)	98.0 (89.4 - 99.2)	84.9	98.7
Total Symptomatic	12.4	24	1	5	3	185	89.3 (71.8 - 97.7)	97.4 (94.0 - 99.1)	82.9	98.5
Site 1 Combined	4.7	15	0	7	2	333	88.2 (63.6 - 98.5)	97.9 (96.4 - 99.5)	67.5	99.4
Site 2 Combined	10.3	11	0	0	2	113	84.6 (54.5 - 98.1)	100 (96.8 - 100)	100	98.3
Site 3 Combined	17.9	41	2	2	2	193	95.3 (84.2 - 99.4)	98.0 (94.9 - 99.4)	91.2	99.0
Total Combined	10.1	67	2	9	6	639	92.0 (85.9 - 98.1)	98.6 (97.4 - 99.4)	88.1	99.1

*Compared to Culture and DFA (95% Confidence Intervals)
**ND: Not Done Unless QuickVue Positive/Culture Negative
***Interpretation of Results as Compared to Culture/DFA:
TP=True Positive; FP=False Positive; FN=False Negative; TN=True Negative;
PPV=Positive Predictive Value; NPV=Negative Predictive Value

Performance Summary: Cytobrush Studies

An additional 500 endocervical specimens, collected on cytology brush, were evaluated in the QuickVue Chlamydia Test at two STD clinical sites. Clinical data are presented below, both before and after resolution of discrepant specimens by DFA analysis. Of the 3 QuickVue positive/culture negative specimens, 1 was found to be positive upon analysis by DFA, indicating that 1/3 QuickVue false positive results was a true positive that was missed by culture.

Cytobrush Performance Summary
Before Discrepant Resolution

	Culture	
	Positive	Negative
QuickVue Positive	46	3
QuickVue Negative	10	441

Sensitivity: 82.1%
(95% Con.Int. 72.1 - 92.2)

Specificity: 99.3%
(95% Con.Int. 98.0 - 99.9)

Accuracy: 97.4%
PPV: 93.7%
NPV: 97.8%

Cytobrush Performance Summary
After Discrepant Resolution

	Culture	
	Positive	Negative
QuickVue Positive	47	2
QuickVue Negative	10	441

Sensitivity: 82.5%
(95% Con.Int. 72.6 - 92.3)

Specificity: 99.5%
(95% Con.Int. 98.4 - 99.9)

Accuracy: 97.6%
PPV: 95.5%
NPV: 97.8%

Analytical Sensitivity

The analytical sensitivity of the QuickVue Chlamydia Test was determined by testing serial dilutions of cultures of known infectivity. The Chlamydia trachomatis serovars A, B, Ba, C, D, E, F, G, I, K, L1 and L3 ranged from ≤ 200 to 2,000 IFU/test; serovars H, L2 and J ranged from 2,000 to 20,000 IFU/test. In addition, C. pneumonia strain TWAR was 300 IFU/test.

Analytical Specificity

A total of 48 culture isolates were evaluated in the test: 32 were organisms that may be isolated from the urogenital tract. Organisms tested at ≥ 10^6 CFU/test produced negative results in the QuickVue Chlamydia Test.

Reproducibility Studies
The within-run, between-run, between-day, between-site performance of the QuickVue Chlamydia Test was evaluated by following the methods contained in the NCCLS EP5-T2 guideline. The studies were conducted at two outside reference laboratories using a blind-coded panel of samples prepared from C. trachomatis Serovar D infected McCoy cell suspensions. The panel contained two low positive samples (150 and 200 IFU/test), two moderately positive samples (500 and 1000 IFU/test) and Positive and Negative Controls. Testing was performed by the laboratory personnel twice per day in replicates of three each level over three days.
All qualitative results obtained at each laboratory site were 100% in agreement with the expected results.

Physician's Office Laboratory (POL) Studies
An evaluation of the QuickVue Chlamydia Test was conducted at three Physician's Offices using a panel of coded specimens. Testing was performed by physician's office personnel with diverse educational backgrounds and work experience at three different locations. The proficiency panel contained blind-coded negative, moderate positive and high positive specimens. Each specimen level was tested in replicates of five at each site over a period of three days. The results obtained at each site ranged from 95-98% agreement with the expected results. No significant differences were observed within run (five replicates), between runs (three different assay days) or between sites (three POL sites).

COMMENTS AND TECHNICAL ASSISTANCE:

If you have any questions regarding the use of this product, please call QUIDEL's Technical Assistance number, 800-874-1517 (U.S. toll-free) or 858-552-1100, Monday through Friday, between 7:00 a.m. and 5:00 p.m., Pacific Time. If outside the United States, contact your local QUIDEL office or distributor.

REFERENCES:

1. U.S. Department of Health and Human Services, Centers for Disease Control, Center for Prevention Services, Division of Sexually Transmitted Diseases. Chlamydia trachomatis infection. Policy guideline for prevention and control. Atlanta: August 1985.

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6. Centers for Disease Control. Update: Universal precautions for prevention of transmission of Human Immunodeficiency virus, Hepatitis B virus, and other blood-borne pathogens in health-care settings. Morbidity and Mortality Weekly Report, Supplement 6/24/88.

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Log Sheet QuickVue® Chlamydia Test

Lot Number: _____
Exp. Date: _____

Record Built-In Positive and Negative Controls. Refer to Positive and Negative Quality Control Section.

	Date	Patient Name	Positive Procedural Control (Blue Line in Control Window)	Negative Procedural Control (Background = no interference)	Test Result at 10 min.	Tech.
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						